ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

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SUMMARY

Methods have been developed for incorporating and recovering B. subtilis var. niger spores in epoxy resin and preliminary data indicate that D values in comparison to Lucite may be twice as great in this type of plastic. A system has been developed for measuring the thermal resistance of spores on stainless steel mated surfaces and preliminary data indicate that D values will be much less for spores so located than for spores encapsulated in Lucite. Measurements of the thermal resistance of spores adjusted to various levels of water activity prior to encapsulation in Lucite indicate that water activity may be a significant consideration in establishing dry heat sterilization processes.

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INTRODUCTION

Efforts during the Seventh Quarter were expended to develop and evaluate the following: a wet grinding system for recovering viable spores from insoluble plastics; a system for adjusting the water activity (a_w) of spores during fabrication of plastic rods and determining the dry heat resistance of such encapsulated spores; a system to determine the dry heat resistance of spores trapped between mated surfaces.

EXPERIMENTAL

Method for Fabricating Inoculated Epoxy Rods

Scotchcast Electrical Resin No. 5 (Minnesota Mining and Manufacturing Company) was used to develop a system for the inoculation and recovery of viable spores from an insoluble plastic. Epoxy shavings, on which an aqueous spore suspension would be inoculated and dried, were produced by disintegrating polymerized plastic on an electrical sanding belt. The solid plastic was prepared by thoroughly mixing Scotchcast Resin Part A (syrup) and Part B (hardner) in a 2:1 ratio. The casting syrup was poured into a beaker coated with Mold Release 225 (Ram Chemicals, Inc.) and allowed 3 hours to polymerize in a water bath at 50° C. The release agent was applied to the mold (beaker) with a cotton applicator and baked at 225°F for 1 hour. The plastic shavings were autoclaved at 15 pounds pressure for 15 minutes and dried overnight

in a forced air oven at 50° C. Sixteen grams of dry, sterile epoxy shavings contained in a sterile drying pan were inoculated with 6.4 milliliters of a stock aqueous suspension of Bacillus subtilis var. niger spores (1 x 10^{11} spores/ml). The pan was placed in a 50° C forced air oven for 30 minutes. The pan was removed and the shavings were placed in a sterile motar and ground by hand until the inoculum appeared to be evenly distributed. The shavings were returned to the drying pan and heated for an additional 30 minutes in the oven followed by a second grinding in a motar. The dry shavings were stored over silica gel in a desiccator for one and one-half hours at room temperature followed by 19 hours at 20° C. The desiccated shavings were added to 64.0 grams of Scotchcast Resin Part A (syrup) and mixed until the shavings appeared to be evenly distributed throughout the resin. Forty-eight grams of Part B (hardner) were added to the inoculated resin and the casting syrup was mixed until it appeared to be homogeneous. The casting syrup was placed into an Erlenmeyer vacuum flask and evacuated with a water pump to remove gas bubbles. The syrup was poured into sterile cotton stoppered thermal-death-time tubes previously coated with Mold Release 225 as described above. The tubes were placed in a 50° C water bath for 3 hours. Following polymerization, the tubes were sealed in an oxy-gas flame. This method consistently produced inoculated epoxy rods that were rigid, unfilled, clear, free of bubbles, and which contained 1 x 10^{8} spores per gram of plastic.

Method for Recovering Viable Spores from Epoxy Rods

Sealed TDT tubes were heated by complete submersion in a silicone bath for various time intervals at the test temperature. Heat treated tubes were cooled in ice water, washed with detergent, and rinsed with distilled water. The tubes were surface disinfected in a saturated iodine solution made up in 70% ethyl alcohol. The tubes were aseptically removed from the alcohol-iodine bath, wiped dry with a sterile paper towel, and scored and opened. The epoxy rod was removed aseptically, placed in a sterile, screw-capped tube, and the tube was weighed. Next, the rod was inserted into the entry port of a modified Waring blendor in which the knives were replaced with a leached 220 grit silicone carbide grinding disk (First Quarterly Report of Progress). Two hundred milliliters of sterile tryptone, glucose, beef extract broth made up to contain 0.0004% Dow Corning Antifoam AF were pipetted into the blendor and a half inch section of the plastic rod was ground by applying a 2000 gram weight to the piston. A dilution plate count of the ground suspension was made using buffered phosphate dilution water as the diluent. All plates were poured with TGE agar made up to contain 5 ml of Tween 80 and 0.7 gram of Asolectin per liter incubated 72 hours at 35° C, counted, and the number of viable spores per gram of plastic was calculated.

Initial experiments at 135° C on the dry heat resistance of B. subtilis var. niger spores encapsulated in epoxy resin by the procedure described above indicate that the D value for spores in epoxy will be greater (about 2.5 hours) than the D value for the same spores in Lucite (1.35 hours).

Method for Controlling the Water Activity of Spores During Fabrication of Plastic Rods

Data presented by Murrell and Scott (1,2) indicate that the dry heat resistance of bacterial spores is related to the water activity (a_w) of the spores. In order to determine the effect of a_w on the dry heat resistance of spores encapsulated in plastic, the following method was devised for incorporating spores of known water activity into plastic.

Lucite powder, in a drying pan, was inoculated and dried at 50° C in a forced air oven as described previously. The pan containing the dry inoculated powder was placed in a desiccator (12 in. diameter) containing 500 milliliters of a saturated salt solution. The desired saturated salt solution required to yield a vapor pressure of known a_w at 25° C in a sealed container, was selected from Robinson and Stokes (3). The saturated solution and excess crystals were poured into the bottom of the desiccator; the pan containing the dry spores was placed on the plate above the solution, and the desiccator was sealed and stored 2 weeks in an incubator operating at 25° C ± 0.5° C. At the end of two weeks, the pan of inoculated powder was removed from the desiccator and the powder was immediately mixed with an equal volume of methyl methacrylate monomer to produce a casting syrup. The subsequent procedure for the fabrication of plastic rods, exposure of the polymerized rods to a dry heat cycle and recovery of viable spores from heated rods is identical to that described in the Fourth Quarterly Report of Progress.

To date, preliminary experiments indicate a marked difference in the dry heat resistance of spores exposed to an a_w of 0.9 and 0.4. Under

conditions of high water activity (0.9), spores appear to be more susceptible to dry heat. The original inoculum of 10^8 spores (a_w = 0.9) per gram of Lucite was reduced to 10^5 spores per gram after 10 minutes exposure at 135° C, whereas, spores adjusted to an a_w = 0.4 required over 2 hours for the original inoculum of 10^8 spores per gram of Lucite to be reduced to 10^5 spores per gram.

Method for Determining the Dry Heat Resistance of Spores Trapped between Mated Surfaces

In order to develop a terminal sterilization cycle, the dry heat resistance of spores trapped between mated surfaces must be established. A unit (figure 1) has been designed and the procedure described below has been developed to establish dry heat sterilization cycles for spores trapped between such surfaces.

Aliquots (0.01 ml) of an aqueous spore suspension was pipetted onto disks fabricated from #302 stainless steel with a No. 4 finish on one face of the disk (C, Fig. 1). The inoculum was placed on that face of the disk with the No. 4 finish and allowed to dry in a forced air oven for 1 hour at 50° C. The dry inoculated disks were stored over silica gel in a dessicator for 1-1/2 hours at room temperature followed by 19 hours at 20° C. The dessicated disks were mounted with the inoculated surface adjacent to the No. 4 finish of a second uninoculated disk (B, Fig. 1) on rod D (Fig. 1). Rods A and D were threaded together to a constant torque of 12 inch pounds. Each mated surface unit was sealed in a sterile TDT tube with an oxy-gas flame. Duplicate samples were exposed for various

time intervals at the test temperature in a silicone bath, cooled, washed, and surface disinfected as described previously.

The tubes were scored and opened and both disks (B and C, Fig. 1) were aseptically placed in a test tube (16 x 150 mm) containing 4.0 milliliters of phosphate buffered dilution water made up to contain 0.0001% Tween-80. The tubes were placed in an ultrasonic bath for 10 minutes after which they were stored at 4° C until assayed. The contents of each tube were mixed 10 seconds in a vortex mixer, and a dilution plate count was made using phosphate buffered dilution water as the diluent. All plates were poured with TGE agar, incubated 24 hours at 35° C, counted and the number of viable spores per disk calculated.

Initial experiments on the recovery of spores from the stainless steel disks indicate that 97-100 percent of the original aqueous inoculum can be recovered. (Table 1).

Dry Heat Resistance of B. subtilis var. niger Spores Encapsulated in Lucite Rods

In the Sixth Quarterly Report of Progress, a value of z_D = 20.8 Centigrade degrees was reported for the slope of the line obtained when the D values for B. subtilis var. niger spores encapsulated in Lucite were plotted against temperature in the range of 105° C to 160° C. The D values for 160° C reported last quarter and used to obtain the z_D values were not corrected for thermal lag which accounted for approximately 55% of the total experimental exposure time. Corrections were made of these data and the following corrected values obtained:

Experiment I, D_{160} = 4.6 min., 95% confidence interval of 4.4 to 4.8 min.; Experiment II, D_{160} = 4.1 min., 95% confidence interval of 3.6 to 4.5 min. These corrected D_{160} values were used for calculating a new regression line for z_D for all the Lucite data obtained at temperatures of 105° C to 160° C. The new value obtained was z_D = 20.7 Centigrade degrees with a 95% confidence interval of 19.3 to 22.1 Centigrade degrees. Though the corrections resulted in only minor changes for the values given in the Sixth Quarterly Report of Progress, the new values listed herein should be substituted for those previously reported in order to avoid confusion in the future.

PROJECTED RESEARCH FOR THE EIGHTH QUARTER

Efforts during the next quarter will be directed toward obtaining D values for B. subtilis var. niger spores encapsulated in epoxy resin and trapped between mated surfaces. In addition, the effect of the water activity (a_w) of spores on their dry heat resistance will be evaluated further.

REFERENCES

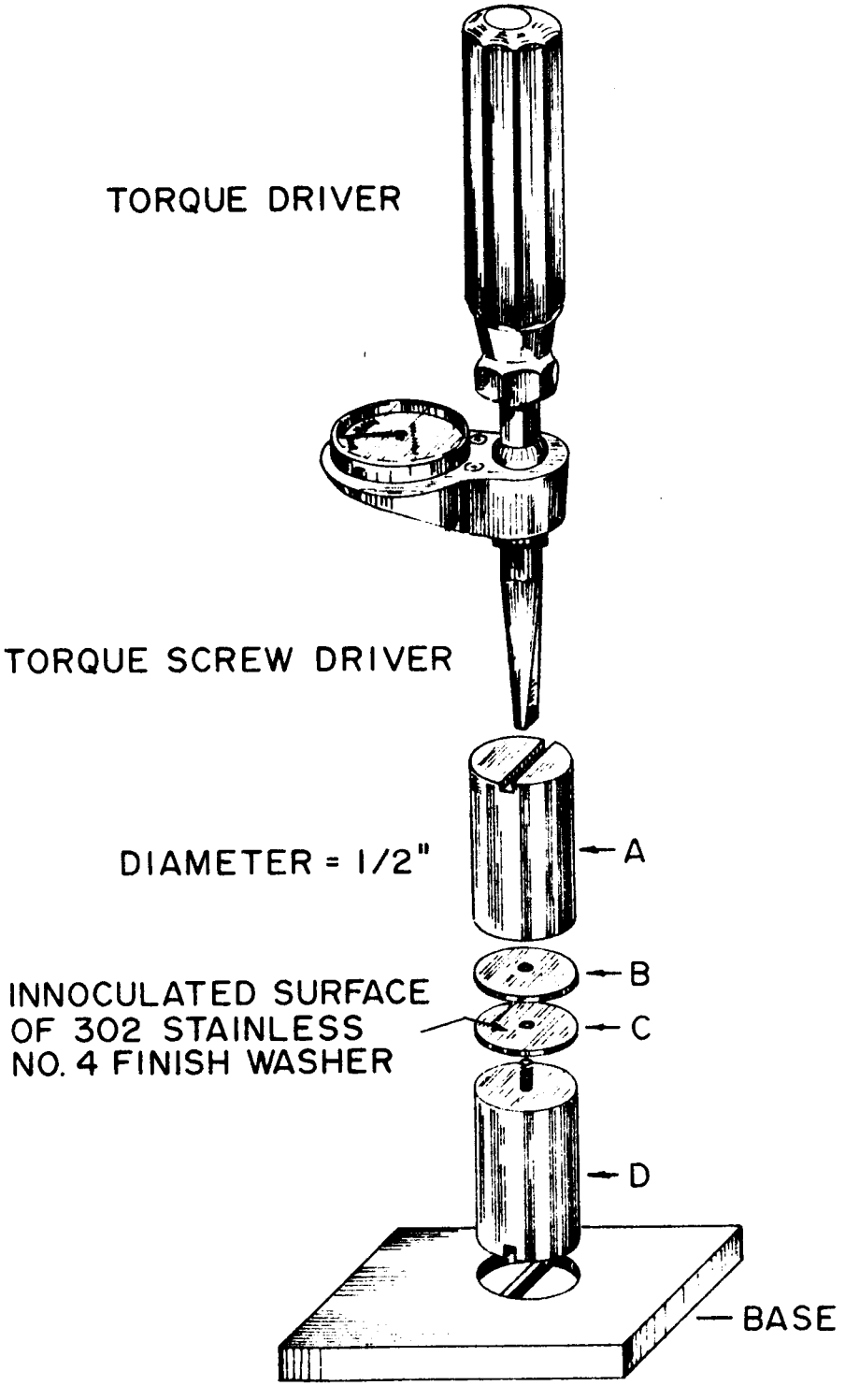
1. Murrell, W. G. and Scott, W. J. Heat resistance of bacterial spores at various water activities. Nature, 179, 481-482, 1957.
2. Murrell, W. G. and Scott, W. J. The heat resistance of bacterial spores at various water activities. J. Gen. Microbiol., 43, 411-425, 1966.
3. Robinson, R. A. and Stokes, R. H. Electrolyte Solutions, 2nd Ed., 1959, Appendix 8.11, p.510, Table 2, Butterworths Publications, England.

TABLE I

	Low Inoculum Level	High Inoculum Level
Aqueous Inoculum	*101	10×10^5
Wet Dish	97	9.9×10^5
Dry Dish	99	9.9×10^5

*Mean No. of spores per disk recovered from ten disks.

Fig. 1



MATED SURFACE ASSEMBLY